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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(b)(2).

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[X] Additional inventors are being named on 1 separately numbered sheets attached hereto.

TITLE OF THE INVENTION (280 characters max)

TUBERCULOSIS VACCINE WITH IMPROVED EFFICACY

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Tuberculosis Vaccine with Improved Efficacy

Specification

The present invention relates to novel recombinant vaccines providing protective immunity especially against tuberculosis.

Tuberculosis (TB) caused by Mycobacterium tuberculosis remains a significant global problem. It is estimated that one third of the world's population is infected with M.tuberculosis (Kochl, 1991). In many countries the only measure for TB control has been vaccination with M.bovis bacille Calmette-Guérin (BCG). The overall vaccine efficacy of BCG against TB, however, is about 50 % with extreme variations ranging from 0 % to 80 % between different field trials (Roche et al., 1995). Thus, BCG should be improved, e.g. by genetic engineering, to provide a vaccine for better TB control (Murray et al., 1996; Hess and Kaufmann, 1993). The widespread emergence of multiple drug-resistant M.tuberculosis strains additionally underlines the urgent requirement for novel TB vaccines (Grange, 1996).

M.tuberculosis belongs to the group of intracellular bacteria that replicate within the phagosomal vacuoles of resting macrophages, thus protection against TB depends on T cell-mediated immunity (Kaufmann, 1993). Several studies in mice and humans, however, have shown that Mycobacteria stimulate antigen-specific, major histocompatibility complex (MHC) class II- or class I-restricted CD4 and CD8 T cells, respectively (Kaufmann, 1993).

The important role of MHC class I-restricted CD8 T cells was convincingly demonstrated by the failure of β2-microglobulin (β2m) deficient mice to control experimental M.tuberculosis infection (Flynn et al., 1993). Because these mutant mice lack MHC class I, functional CD8 T cells cannot

develop. In contrast to M.tuberculosis infection, β2m-deficient mice are capable of controlling certain infectious doses of the BCG vaccine strain (Flynn et al., 1993; Ladel et al., 1995). Furthermore, BCG vaccination of β2m-deficient mice prolonged survival after subsequent M.tuberculosis infection whereas BCG-immunized C57BL/6 resisted TB (Flynn et al., 1993). This differential CD8 T cell dependency between M.tuberculosis and BCG may be explained as follows: M.tuberculosis antigens gain better access to the cytoplasm than antigens from BCG leading to more pronounced MHC class I presentation (Hess and Kaufmann, 1993). Consequently, a more effective CD8 T cell response is generated by M.tuberculosis. This notion was recently supported by increased MHC class I presentation of an irrelevant antigen, ovalbumin, by simultaneous M.tuberculosis, rather than BCG, infection of antigen presenting cells (APC) (Mazzaccaro et al., 1996).

Secreted proteins of M.tuberculosis comprise a valuable source of antigens for MHC class I presentation. Recently, a DNA vaccine encoding the secreted antigen Ag85A elicited MHC class I-restricted CD8 T cell responses in mice which may contribute to defence against TB (Huygen et al., 1996). In general, evidence is accumulating that immunization with secreted protein antigens of M.tuberculosis induce some protection against TB in guinea pigs and mice (Horwitz et al., 1995; Andersen, 1994). An important goal towards the development of improved TB vaccines based on BCG, therefore, is to augment the accessibility of secreted BCG-specific antigens to the cytoplasm of infected APC. Subsequent delivery of peptides derived from these secreted proteins into the MHC class I presentation pathway may potentiate the already existing BCG-specific immune response for preventing TB.

The phagolysosomal escape of L.monocytogenes represents a unique mechanism to facilitate MHC class I antigen presentation of listerial antigens (Berche et al., 1987; Portnoy et al., 1988). Listeriolysin (Hly), a

pore-forming sulfhydryl-activated cytolysin, is essential for the release of L.monocytogenes microorganisms from phagolysosomal vacuoles into the cytosol of host cells (Gaillard et al., 1987; Portnoy et al., 1988). This escape function was recently transferred to Bacillus subtilis and to attenuated Salmonella ssp. strains (Bielecki et al., 1991; Gentschev et al., 1995; Hess and Kaufmann, 1997). Hly expression by an asporogenic B.subtilis mutant strain or in Salmonella ssp. results in bacterial escape from the phagolysosome into the cytosol of J774 macrophage-like cells (Bielecki et al., 1991; Gentschev et al., 1995; Hess and Kaufmann, 1997).

WO 99/101496 and Hess et al. (1998) disclose recombinant Mycobacterium bovis strains that secrete biologically active Listeriolysin fusion proteins. These M.bovis strains have been shown to be effective vaccines against TB in several animal models.

According to the present invention Hly was expressed in urease-deficient BCG strains. These urease-deficient BCG strains exhibit an increased Hly activity in phagosomes and in turn improved pore formation in the endosomal membranes leading to superior immunoprotectivity.

Thus, a first aspect of the present invention is a bacterial cell, particularly a Mycobacterium cell which is urease-deficient and comprises a recombinant nucleic acid molecule encoding a fusion polypeptide comprising (a) at least one domain from a polypeptide, wherein said polypeptide domain is capable of eliciting an immune response in a mammal, and (b) a phagolysosomal escape domain. It is preferred that the cell is capable of expressing the nucleic acid molecule of the invention. More preferably, the cell is capable of secreting the fusion polypeptide and/or of providing it in a form suitable for MHC class I-restricted antigen recognition.

The bacterial cell of the invention is a urease-deficient cell, e.g. a gram-negative or a gram-positive bacterial cell, preferably a Mycobacterium cell. The urease-deficiency may be achieved by partially or completely inactivating one or several cellular nucleic acid molecules which code for a urease subunit, particularly ureA encoding for urease subunit A, ureB coding for urease subunit B and/or ureC coding for urease subunit C. The sequences of ureA, ureB and ureC in Mycobacteria, particularly M.bovis and M.tuberculosis and the proteins encoded thereby are described by Reyrat et al. (1995) and Clemens et al. (1995), which are incorporated herein by reference.

Preferably the urease-deficient bacterial strain is obtained by deletions and/or insertions of one or several nucleotides in urease subunit - coding nucleic acid sequences and/or their expression control sequences. Deletions and/or insertions may be generated by homologous recombination, transposon insertion or other suitable methods.

In an especially preferred embodiment the ureC sequence is inactivated, e.g. by constructing a suicide vector containing a ureC gene disrupted by a selection marker gene, transforming the target cell with the vector and screening for selection marker-positive cells having a urease negative phenotype as described by Reyrat et al. (1995).

The cell of the invention is preferably an M.bovis cell, a M.tuberculosis cell, particularly an attenuated M.tuberculosis cell or other Mycobacteria, e.g. M.microti, M.smegmatis, M.canettii, M.marinum or M.fortuitum or Mycobacteria as described by Reyrat et al. (1995).

The Mycobacterium cell of the invention comprises a recombinant nucleic acid molecule, e.g. the nucleic acid molecule in SEQ ID No.1. This nucleic acid molecule comprises a signal peptide coding sequence (nucleotide 1 - 120), a sequence coding for an immunogenic domain (nucleotide 121 -

153), a peptide linker coding sequence (nucleotide 154 - 210), a sequence coding for a phagolysosomal domain (nucleotide 211 - 1722), a further peptide linker coding sequence (nucleotide 1723 - 1800) and a sequence coding for a random peptide (nucleotide 1801 - 1870). The corresponding amino acid sequence is shown in SEQ ID No.2.

The nucleic acid contains at least one immunogenic domain from a polypeptide. The immunogenic domain may be derived from an organism of the genus Mycobacterium, preferably from Mycobacterium tuberculosis or from Mycobacterium bovis. This domain has a length of at least 6, preferably of at least 8 amino acids. The immunogenic domain is preferably a portion of a native Mycobacterium polypeptide. However, within the scope of the present invention is also a modified immunogenic domain, which is derived from a native immunogenic domain by substituting, deleting and/or adding one or several amino acids.

The immunogenic domain is however not restricted to Mycobacterium antigens and can be selected from autoantigens, tumor antigens and pathogen antigens such as virus antigens, parasite antigens, bacterial antigens in general and immunogenic fragments thereof. Specific examples for suitable tumor antigens are human tumor antigens such as the p53 tumor suppressor gene product (Houbiers et al., 1993) and melanocyte differentiation antigens, e.g. Melan-A/MART-1 and gp100 (van Elsas et al., 1996). Specific examples for suitable virus antigens are human tumor virus antigens such as human papilloma virus antigens, e.g. antigens E6 and E7 (Bosch et al., 1991), influenza virus antigens, e.g. influenza virus nucleoprotein (Matsui et al., 1995; Fu et al., 1997) or retroviral antigens such as HIV antigens, e.g. the HIV-1 antigens p17, p24, RT and Env (Harrer et al., 1996; Haas et al., 1996). Specific examples for suitable parasite antigens are Plasmodium antigens such as liver stage antigen (LSA-1), circumsporozoite protein (CS or allelic variants cp26 or cp29), thrombospondin related amonymous protein (TRAP), sporozoite threonine

and asparagine rich protein (STARP) from Plasmodium falciparum (Aidoo et al., 1995) and Toxoplasma antigens such as p30 from Toxoplasma gondii (Khan et al., 1991; Bulow and Boothroyd, 1991). Specific examples for suitable bacterial antigens are Legionella antigens such as Major secretary protein from Legionella pneumophila (Blander and Horwitz, 1991).

The immunogenic domain is capable of eliciting an immune response in a mammal. This immune response can be a B cell-mediated immune response. Preferably, however, the immunogenic domain is capable of eliciting a T cell-mediated immune response, more preferably a MHC class I-restricted CD8 T cell response.

The domain capable of eliciting an immune response is more preferably selected from immunogenic peptides or polypeptides from M.bovis or M.tuberculosis or from immunogenic fragments thereof. Specific examples for suitable antigens are Ag85B (p30) from M.tuberculosis (Harth et al., 1996), Ag85B (α-antigen) from M.bovis BCG (Matsuo et al., 1988), Ag85A from M.tuberculosis (Huygen et al., 1996) and ESAT-6 from M.tuberculosis (Sorensen et al., 1996, Harboe et al., 1996 and Andersen et al., 1995). More preferably, the immunogenic domain is derived from the antigen Ag85B. Most preferably, the immunogenic domain comprises the sequence from aa.41 to aa.51 in SEQ ID No.2.

The recombinant nucleic acid molecule according to the present invention further comprises a phagolysosomal escape domain, i.e. a polypeptide domain which provides for an escape of the fusion polypeptide from the phagolysosome into the cytosol of mammalian cells. Preferably, the phagolysosomal escape domain is a Listeria phagolysosomal escape domain, which is described in US 5,733,151, herein incorporated by reference. More preferably, the phagolysosomal escape domain is derived from the organism L.monocytogenes. Most preferably, the phagolysosomal domain is encoded by a nucleic acid molecule selected from: (a) a

nucleotide sequence comprising nucleotides 211 - 1722 as shown in SEQ ID No.1, (b) a nucleotide sequence which encodes for the same amino acid sequence as the sequence from (a), and (c) a nucleotide sequence hybridizing under stringent conditions with the sequence from (a) or (b).

Apart from the nucleotide sequence depicted in SEQ ID No.1 the present invention also comprises nucleic acid sequences hybridizing therewith. In the present invention the term "hybridization" is used as defined in Sambrook et al. (Molecular Cloning. A laboratory manual, Cold Spring Harbor Laboratory Press (1989), 1.101-1.104). In accordance with the present invention the term "hybridization" is used if a positive hybridization signal can still be observed after washing for one hour with 1 X SSC and 0.1 % SDS at 55°C, preferably at 62° C and more preferably at 68°C, particularly for 1 hour in 0.2 X SSC and 0.1 % SDS at 55°C, preferably at 62°C and more preferably at 68°C. A sequence hybridizing with a nucleotide sequence as per SEQ ID No.1 under such washing conditions is a phagolysosomal escape domain encoding nucleotide sequence preferred by the subject invention.

A nucleotide sequence encoding a phagolysosomal escape domain as described above may be directly obtained from a Listeria organism or from any recombinant source e.g. a recombinant E.coli cell containing the corresponding Listeria nucleic acid molecule or a variant thereof as described above.

Preferably, the recombinant nucleic acid molecule encoding for a fusion polypeptide contains a signal peptide encoding sequence. More preferably, the signal sequence is a signal sequence active in Mycobacteria, preferably in M.bovis, e.g. a native M.bovis signal sequence. A preferred example of a suitable signal sequence is the nucleotide sequence coding for the Ag85B signal peptide which is depicted in SEQ ID No.1 from nucleotide 1 to 120.

Further, it is preferred that a peptide linker be provided between the immunogenic domain and the phagolysosomal escape domain. Preferably, said peptide linker has a length of from 5 to 50 amino acids. More preferably, a sequence encoding a linker as shown in SEQ ID No.1 from nucleotide 154 to 210 or a sequence corresponding thereto as regards the degeneration of the genetic code.

The nucleic acid may be located on a recombinant vector. Preferably, the recombinant vector is a prokaryotic vector, i.e. a vector containing elements for replication or/and genomic integration in prokaryotic cells. Preferably, the recombinant vector carries the nucleic acid molecule of the present invention operatively linked with an expression control sequence. The expression control sequence is preferably an expression control sequence active in Mycobacteria, particularly in M.bovis. The vector can be an extrachromosomal vector or a vector suitable for integration into the chromosome. Examples of such vectors are known to the man skilled in the art and, for instance, given in Sambrook et al. supra.

In a further aspect of the present invention a urease-deficient bacterial cell e.g., a Mycobacterium cell, preferably an M.bovis cell is provided which comprises at least one nucleic acid molecule encoding a phagolysosomal escape peptide or polypeptide. Even if the phagolysosomal escape peptide or polypeptide is not fusioned with an antigen, a surprising improvement of the immunogenic properties is found.

The recombinant bacterial cell which is provided according to this further ascpect of the present invention may contain at least one further recombinant, e.g. heterologous nucleic acid molecule encoding a peptide or polypeptide capable of eliciting an immune response in a mammal. Said further immunogenic peptide or polypeptide may be selected from Mycobacterium antigens or, in a wider sense, from autoantigens, tumor antigens, pathogen antigens and immunogenic fragments thereof. The

nucleic acid molecule coding for the further peptide or polypeptide may be situated on the same vector as the fusion gene. However, it may, for example, also be situated on a different plasmid, independently of the fusion gene, or be chromosomally integrated.

Surprisingly, it was found that a Mycobacterium cell according to the present invention has an intracellular persistence in infected cells, e.g. macrophages, which is equal or less than the intracellular persistence of a corresponding native Mycobacterium cell which does not contain the recombinant nucleic acid molecule.

The present invention also refers to a pharmaceutical composition comprising as an active agent a cell as defined above, optionally together with pharmaceutically acceptable diluents, carriers and adjuvants. Preferably, the composition is a living vaccine suitable for administration to a mammal, preferably a human. The actually chosen vaccination route depends on the choice of the vaccination vector. Administration may be achieved in a single dose or repeated at intervals. The appropriate dosage depends on various parameters such as the vaccinal vector itself or the route of administration. Administration to a mucosal surface (e.g. ocular, intranasal, oral, gastric, intestinal, rectal, vaginal or urinary tract) or via the parenteral route (e.g. subcutaneous, intradermal, intramuscular, intravenous or intraperitoneal) might be chosen.

Further, the present invention pertains to a method for preparing a recombinant bacterial cell as defined above. According to the first aspect, this method comprises the steps of (i) providing a urease-deficient bacterial cell, particularly a Mycobacterium cell, (ii) inserting a recombinant nucleic acid molecule into said bacterial cell, said nucleic acid molecule encoding a fusion polypeptide comprising (a) at least one domain from a polypeptide wherein said domain is capable of eliciting an immune response in a mammal and (b) a phagolysosomal escape domain, and (iii) cultivating the

cell obtained according to step (ii) under suitable conditions. Preferably, a cell is obtained which is capable of expressing said nucleic acid molecule. More preferably, the cell is an M.bovis cell.

According to the further aspect, this method comprises the step of (i) providing an urease-deficient bacterial cell, particularly a Mycobacterium cell, (ii) inserting a recombinant nucleic acid molecule into said bacterial cell, said nucleic acid molecule encoding a phagolysosomal escape peptide or polypeptide, and (iii) cultivating the cell obtained according to (ii) under suitable conditions.

If desired, the method of the present invention comprises inserting at least one further recombinant nucleic acid molecule into the bacterial cell, said further recombinant nucleic acid molecule encoding a peptide or polypeptide capable of eliciting an immune response in a mammal.

Finally, the present invention relates to a method for the preparation of a living vaccine comprising formulating the recombinant cell in a pharmaceutically effective amount with pharmaceutically acceptable diluents, carriers and/or adjuvants.

The invention will be further illustrated by the following figures and sequence listings.

Fig.1: the protective capacity of rΔureC BCG Hly in the aerosol model of murine tuberculosis. BALB/c mice were immunized i.v. with $1x10^6$ CFU rΔureC BCG Hly or BCG "Pasteur". 120 days post vaccination animals were challenged with H37Rv (200 organism/lung) via aerosol. Bacterial load in infected organs (spleen and lung) was assessed 30, 60 and 90 days post challenge. Each bar represents 10 animals.

SEQ ID No.1: shows the nucleotide sequence of a nucleic acid molecule according to the present invention.

SEQ ID No.2: shows the corresponding amino acid sequence of the nucleic acid molecule of SEQ ID No.1.

Example

1. Inactivation of the urease activity of BCG delta ureC.

To obtain a urease-deficient mutant, Reyrat et al. constructed a suicide vector containing a ureC gene disrupted by a kanamycin marker (the aph gene). Two micrograms of this construct were linearized with Sac I and electroporated into M. bovis BCG. Kanamycine resistant transformants were screened for urease negative phenotype (cf. Reyrat et al., 1995).

2. Construction of the mycobacterial E. coli shuttle expression vector pMV306:Hly.

To transfer the phagosomal escape function (mediated by Hly of L. monocytogenes EGD Sv 1/2a), to BCG Pasteur (1173 P_3) delta ureC, an E. coli-mycobacterial shuttle vector was used. The intergrative plasmid pMV306, a precursor of vector pMV361, allows stable chromosomal expression of Hly.

A pILH-1-derived 1.7-kb PstI DNA fragment coding for an hly-hlyA (E. coli pHly152-specific hemolysin A) ORF was inserted into PstI site of plasmid pAT261. This resulting gene fusion codes for the expression of secreted proteins directed to the supernatant by the BCG-specific Ag85B signal peptide. The construct was termed pAT261:Hly and its XbaI-SalI DNA expression cassette under

transcriptional control of the hsp60 mycobacterial promoter was subsequently used for insertion into the parental pMV306 vector resulting in the construct pMV306:Hly. The DNA sequence of the hly-specific insertion sites in both mycobacterial expression plasmids was analyzed. The mature Hly fusion protein putatively consists of 30 aa at the N terminus and 52 aa at the C-terminal part of the fusion that partially belong to HlyA of E. coli.

3. Protective capacity

The expression vector pMV306:Hly was transformed into an urease deficient BCG strain. The resultant strain was designated r ΔrureC/BCG Hly. The protective capacity of this urease-deficient mycobacterial strain in a model of murine tuberculosis is shown in Figure 1.

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Claims

1. A bacterial cell which is urease-deficient and which comprises a recombinant nucleic acid molecule encoding a fusion polypeptide comprising (a) at least one domain from a polypeptide, wherein said polypeptide domain is capable of eliciting an immune response in a mammal, and (b) a phagolysosomal escape domain.

2. The cell of claim 1 wherein at least one cellular urease subunit encoding nucleic acid sequence is inactivated.

3. The cell of claim 2 wherein at least the cellular urease C subunit-encoding sequence is inactivated.

4. The cell of claim 1, wherein said phagolysosomal escape domain is a Listeria phagolysosomal escape domain.

5. The cell of claim 1, wherein said phagolysosomal domain is encoded by a nucleic acid molecule selected from:
   (a) a nucleotide sequence comprising nucleotide 211 - 1722 as shown in SEQ ID No.1;
   (b) a nucleotide sequence which encodes for the same amino acid sequence as the sequence from (a), and
   (c) a nucleotide sequence hybridizing under stringent conditions with the sequence from (a) or (b).

6. The cell of claim 1, wherein the domain capable of eliciting an immune response is a peptide or polypeptide capable of eliciting MHC class I-restricted CD8 T cell responses.

7. The cell of claim 1 wherein the domain capable of eliciting an immune response is from a Mycobacterium polypeptide.

8. The cell of claim 7, wherein the domain capable of eliciting an immune response is selected from the Mycobacterium antigens Ag85B (M.tuberculosis), Ag85B (M.bovis), Ag85A (M.tuberculosis) and ESAT-6 (M.tuberculosis) or an immunogenic fragment thereof.

9. The cell of claim 8, wherein the domain capable of eliciting an immune response is the antigen Ag85B or an immunogenic fragment thereof.

10. The cell of claim 1, wherein the fusion polypeptide is preceded by a signal peptide sequence.

11. The cell of claim 1, wherein a peptide linker is located between the immune response eliciting domain and the phagolysosomal domain.

12. The cell of claim 1, wherein said nucleic acid molecule is operatively linked with an expression control sequence.

13. The cell of claim 12, wherein said expression control sequence is active in said cell.

14. The cell of claim 1 wherein said nucleic acid molecule is located on a vector.

15. The cell of claim 1 which is a Mycobacterium cell.

16. The cell of claim 16 which is a Mycobacterium bovis cell.

17. A bacterial cell which is urease-deficient and which comprises at least one recombinant nucleic acid molecule encoding a phagolysosomal escape peptide or polypeptide.

18. The cell of claim 17, which comprises at least one further recombinant nucleic acid molecule encoding a peptide or polypeptide capable of eliciting an immune response in a mammal.

19. The cell of claim 18 which is a Mycobacterium cell.

20. The cell of claim 19 which is a Mycobacterium bovis cell.

21. The cell of claims 1 or 17, wherein the domain or peptide or polypeptide capable of eliciting an immune response is selected from autoantigens, tumor antigens, virus antigens, parasite antigens, bacterial antigens and immunogenic fragments thereof.

22. The cell of claims 1 or 17, which is capable of expressing said at least one recombinant nucleic acid molecule.

23. The cell of claim 22, which is capable of secreting a polypeptide encoded by said at least one nucleic acid molecule.

24. The cell of claims 1 or 23, which has an intracellular persistence in infected macrophages which is equal or less than the intracellular persistence of a native Mycobacterium cell.

25. A pharmaceutical composition comprising as an active agent a cell of claims 1 or 17, optionally together with pharmaceutically acceptable diluents, carriers and adjuvants.

26. The composition of claim 25, which is a living vaccine suitable for administration to a mucosal surface or via the parenteral route.

27. A method for the preparation of a living vaccine comprising formulating a cell of claims 1 or 17 in a pharmaceutically effective amount with pharmaceutically acceptable diluents, carriers and adjuvants.

28. A method for preparing a recombinant bacterial cell of claim 1 comprising the steps:

(i) providing a urease-deficient bacterial cell;

(ii) inserting a recombinant nucleic acid molecule into said bacterial cell, said nucleic acid molecule encoding a fusion polypeptide comprising (a) at least one domain from a polypeptide, wherein said domain is capable of eliciting an immune response in a mammal, and (b) a phagolysosomal escape domain, and

(iii) cultivating the cell obtained according to (ii) under suitable conditions.

29. The method of claim 28, wherein said cell is a M.bovis cell.

30. A method for preparing a recombinant bacterial cell of claim 17 comprising the steps:

(i) providing a urease-deficient bacterial cell;

(ii) inserting a recombinant nucleic acid molecule into said bacterial cell, said nucleic acid molecule encoding a phagolysosomal escape peptide or polypeptide and

(iii) cultivating the cell obtained according to (ii) under suitable conditions.

31. The method of claim 30 comprising inserting at least one further recombinant nucleic acid molecule into the bacterial cell, said further recombinant nucleic acid molecule encoding a peptide or polypeptide capable of eliciting an immune response in a mammal.

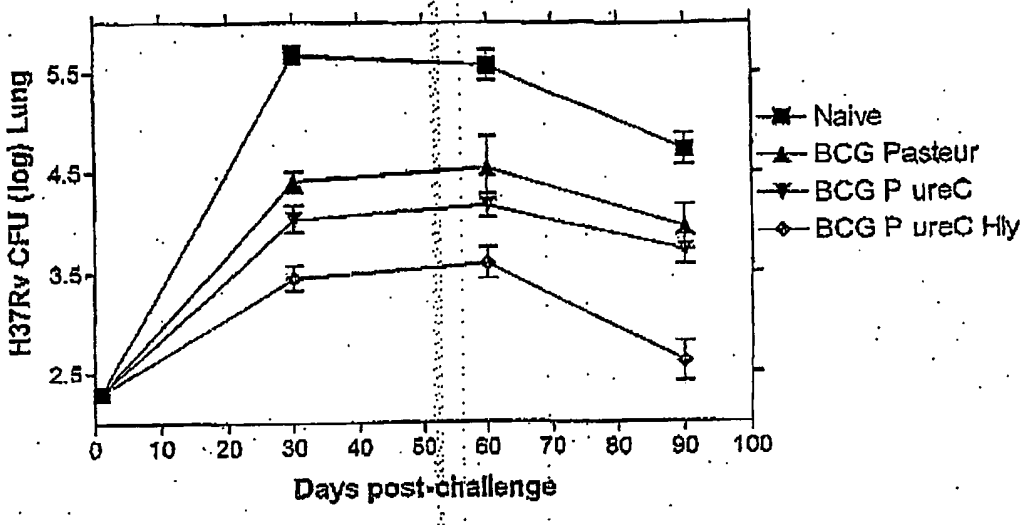
32. The method of claim 28 or 30, wherein the domain or peptide or polypeptide capable of eliciting an immune response is selected from autoantigens, tumor antigens, virus antigens, parasite antigens, bacterial antigens and immunogenic fragments thereof.

Abstract

The present invention relates to novel recombinant vaccines providing protective immunity against tuberculosis. Further, the present invention refers to novel recombinant nucleic acid molecules, vectors containing said nucleic acid molecules, cells transformed with said nucleic acid molecules and polypeptides encoded by said nucleic acid molecules.

Attorney Dkt # 2001-6586.04-042303
Grode, et al.

Figure 1



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Max-Planck-Gesellschaft zur Foerderung der Wissenschaften e.V.
(B) STREET: Hofgartenstrasse 2
(C) CITY: Muenchen
(E) COUNTRY: Germany
(F) POSTAL CODE (ZIP): 80539

(ii) TITLE OF INVENTION: Tuberculosis vaccine

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1881 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1878

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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TAT ATC TCA AGT GTG GCG TAT GGC CGT CAA GTT TAT TTG AAA TTA TCA     1056
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(2) INFORMATION FOR SEQ ID NO: 2:

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(ii) MOLECULE TYPE: protein
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Ser Thr
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